NOTE TO THE FILE
BNF0050
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Subject: Sulfonylurea Tolerant Linseed Flax

Keywords:
Flax (*Linum usitatissimum*), Sulfonylurea herbicide tolerant, *Arabidopsis* acetolactate synthase *(csr-1)* gene, Acetolactate synthase (ALS protein), , kanamycin resistance (*nptII*) gene, neomycin phosphotransferase II (NPTII protein), *Agrobacterium* nopaline synthase (*nos*) gene, Nopaline synthase (NOS protein).

Background
In submissions dated October 27, 1997 and February 6 and 10, 1998, Dr. A. McHughen of the University of Saskatchewan provided summary information to support the safety and nutritional assessment of new linseed flax variety, CDC Triffid, also known as line 12115 or FP967.

Intended Effect and Food/Feed Use
According to the developer, the intended technical effect of these genetic modifications of linseed flax is to confer tolerance to soil residues of sulfonylurea herbicides. These residues commonly occur when farmers treat cereal crops with sulfonylurea herbicides which can have a long soil half-life. These residues inhibit the growth of flax and other crops and prevent the use of agronomically beneficial crop rotation sequences. The developer reports that insertion and expression of the modified *csr-1* gene confers sulfonylurea tolerance, since the expressed enzyme is not inhibited by the herbicide residues.

The submission states that the *csr-1* gene present in Triffid flax was isolated from a mutant line of *Arabidopsis*. The alteration consists of a single base modification which results in a change of one amino acid in the expressed protein. This substitution prevents enzyme inhibition by the herbicide. The submission indicates that flax has homologous ALS genes, but these enzymes can be inhibited by sulfonylurea herbicides. Nopaline synthase (NOS), the expression product of the *nos* gene, was initially used as a scorable marker in the 1980s, but this use is now considered obsolete. The *nos* gene was obtained from the *Agrobacterium tumefaciens* Ti plasmid pTiC38. The *nptII* gene was obtained from the *E. coli* transposon Tn5 and confers resistance to some aminoglycoside antibiotics including neomycin and kanamycin, and was used by the developers of the variety as a selectable marker for transformed plants cells.

The developer reports that flax is most often used for the industrial production of linseed oil. However, the residue from this process, flax or linseed meal, is often utilized as an animal feedstuff. The submission indicates that transgenic flax grown in Canada will most likely be exported to the United States for crushing or extraction of the oil. The resultant meal will be used in the United States as a feed ingredient. The developer reports that little flaxseed is consumed by humans. It is occasionally eaten as a health food in a whole or ground state.

The Flax Council of Canada is reported to estimate annual human consumption of flaxseed to be approximately 4,000 tons.

Molecular Alterations and Characterization

The novel genetic material contained in Triffid flax was inserted into the commercial cultivar, NorLin, using *Agrobacterium tumefaciens*-mediated transformation. The T-DNA contained the following genes with eukaryotic regulatory sequences: *csr-1*, *nos*, and *nptII*. The *csr-1* gene confers herbicide tolerance, while the *nos* and *nptII* genes were used as selection aids to identify modified plant cells. The remainder of the T-DNA is composed of prokaryotic genes coding for ampicillin (*amp*) and streptomycin/spectinomycin (*spc/str*) resistances.

The modified cultivar has been studied for eight seed generations. Both genetic and molecular analyses of the progeny are reported to indicate that there are two stable, functional, unlinked inserts in the flax line. One insert contains the left T-DNA border and the gene coding for ALS and thus, confers herbicide tolerance. The second insert has one copy of *nptII* and two copies of *nos* and T-DNA right border. The first insert also contains the *amp* gene, while the *spc/str* gene is present in the second insert. The developer reports that both these antibiotic resistance genes are under the control of prokaryotic regulatory sequences and thus, would not be expected to be expressed in the flax.

Expressed Proteins/ Regulatory Considerations

The ALS protein, acetolactate synthase, catalyzes an intermediate step in the syntheses of the amino acids, valine, isoleucine, and leucine. The developer reports that the expression product of the inserted genetic material has the same functional activity of the naturally occurring enzyme and is not expected to alter plant composition. Levels of ALS protein were determined indirectly by measuring the amount of ALS enzymatic activity present in the parent and in the modified variety. Acetolactate activity was 56.3 nmol/mg/hr for the parent cultivar and 88.8 nmol/mg/hr for the modified variety. The developer attributes the increase in activity to the aggregate activity of the endogenous and inserted ALS enzymes. The submission notes that the increase in enzymatic activity did not lead to increased levels of the end-product amino acids.

Neomycin phosphotransferase II, also known as aminoglycoside 3'-phospho-transferase II, is regulated as a food additive under 21 CFR 173.150 and 573.130 for use as a processing aid in the development of new varieties of tomato, oilseed rape and cotton. FDA evaluated NPTII as a food additive in response to a petition filed by Calgene, Inc. At the time the petition was filed, the use of NPTII as a processing aid in the development of new plant varieties was new and a record of safe use in food for human and animal consumption had not yet been established, nor had its use in food or feed been evaluated. Since then, scientific studies and evaluations regarding the use of NPTII in new plant development have been conducted. In FDA's review of NPTII, FDA concluded that NPTII will not compromise the efficacy of antibiotic treatment, the probability of transfer of the *nptII* (*kan*^{r}) gene consumed as a component of crops to microorganisms in the gastrointestinal tract is remote, and NPTII

does not have any properties that would distinguish it toxicologically from any other phosphorylating enzyme in the food supply.

The *nptII* gene is expressed in the flax and levels of NPTII protein in Triffid seed, as determined using an ELISA assay, are 432 pg/ mg total protein or 0.000043% of the total protein. The submission states that no active NPTII is expected to be present in flaxseed meal fed to animals due to the nature of the processing that the seed undergoes. The developer previously indicated that human consumption of flaxseed is extremely minor, and thus, the estimated dietary exposure to NPTII will not be substantially increased over the current levels of consumption. The developer states that "There is no indication that the gene or its product in Triffid poses any greater risk to human or animals health than it does in tomato, potato, cotton or canola." It is FDA's understanding that, in essence, the developer has concluded that the use of NPTII as a processing aid in the development of Triffid transgenic flax is safe and has provided information regarding the safety of NPTII with its safety assessment.

Nopaline synthase, NOS, converts arginine to nopaline and was used as a scorable marker in early transformation experiments. However, due to problems with false positive results because some plants naturally produce nopaline, this use is considered obsolete. The developer reports that nopaline is present in the vegetative portions of the flax plant (leaf, stem, root), but is not detectable in the seed which it reports is the only portion of the plant consumed by man or other animals. The reported detection limit of the assay is 0.025 mg opine/ mg fresh tissue. The presence of the enzyme indicates that the *nos* gene is expressed in these parts of the plant.

The developer indicates that none of the introduced genes or their expression products present in Triffid flax have a history of allergenic concern. It reports that computer searches of various databases, including the Kabat database which contains amino acid sequences with immunological significance, for the introduced genes and their expression products did not reveal any significant homology with immunologically significant sequences.

The submission also states that the cultivar has received approval in Canada for both human food and animal feed uses and that it has clearance for both environmental and agronomic concerns.

Nutritional Assessment

The developer reports that only the grain is consumed by man or other animals and thus, conducted analytical measures on whole seed. The intent of the genetic modification was to confer herbicide tolerance and the developer did not anticipate any unintended effects from the introduction of the novel genetic material. However, to confirm this assumption, compositional analyses were conducted and included the proximate analyses (protein, moisture, oil, ether extract fat, crude fiber, and ash) and amino acid composition. The seed was also analyzed for its content of nitrogen, potassium, phosphorus, sulfur, calcium,

magnesium, copper, iron, manganese, zinc, and boron. The developer reports that there were no significant differences between the modified cultivar and its patent, NorLin, except for ether extract fat where the modified variety contained slightly higher levels. However, the developer concludes that there are no substantive differences in composition and that all the results are within the normal range observed in flax.

The developer reports that flax produces cyanogenic glycosides. Concentrations of these compounds are reported to vary with year, planting location, and cultivar. Environmental conditions also play a major role in determining the final concentrations of these compounds. The developer states that the typical range for Canadian cultivars, including NorLin, is 6.0 to 9.2 mg/g seed. The submission reports that the modified variety contains 7.7 mg/g seed which falls within the normal range for commercial varieties and does not differ significantly from values obtained with the parent. Additional tests conducted using a more sensitive assay, found nonsignificant differences between the concentrations of the cyanogenic glycosides in Triffid (7.2 mg/g), and its parent (7.01 mg/ g).

Overall, the developer concludes that there are no substantive compositional differences between Triffid and its parent and that all values fall within ranges ordinarily seen for flax.

Conclusions

The developer has concluded, based on its safety and nutritional assessment, that flax modified to be tolerant to sulfonylurea herbicides is not materially different in composition, safety, or any relevant parameter from flax now grown, marketed, and consumed. At this time, based on the developer's description of the data and analysis, the Agency considers the consultation on CDC Triffid flax to be complete.

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